

SHORT TERM SUBLETHAL STUDIES IN RATS EXPOSED  
TO NICKEL SUBSULFIDE AND NICKEL ORE:  
EFFECTS ON OXIDATIVE DAMAGE, ANTIOXIDANT  
AND DETOXICATING ENZYMES

CENTRE FOR NEWFOUNDLAND STUDIES

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**Short Term Sublethal Studies in Rats Exposed to Nickel Subsulfide  
and Nickel Ore: Effects on Oxidative Damage, Antioxidant  
and Detoxicating Enzymes**

by

© Xiangning Chang, B. Sc

A thesis submitted to the School of Graduate  
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Memorial University of Newfoundland  
St. John's, Newfoundland

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## ABSTRACT

Nickel is a ubiquitous trace metal which occurs in soil, water, air, and in the biosphere. Some nickel compounds have been shown to induce tumor at injection site in animals. Lung and nasal cancers have been observed in workers at nickel refineries. The mining of nickel in Voisey's Bay may possibly pose risks of environmental concern, especially to aquatic species. The sublethal effects of nickel subsulfide and nickel ore with respect to oxidative damage, antioxidant and detoxicating enzymes were examined in short term studies in rats.

The rats were given nickel subsulfide (100 mg/rat or 200 mg/rat), i.p., as a single dose or two doses, 2 weeks prior to sacrifice. Nickel subsulfide suppressed catalase and superoxide dismutase (SOD) activities in rat liver but not in kidney. The inhibition in hepatic SOD showed a dose-dependent relationship. The decrease of hepatic catalase and SOD by nickel subsulfide could be a reflection of nickel insult which could make target tissues more vulnerable under oxidative stress.

Other groups of rats were given nickel ore (100, 200 mg/rat), i.p., as a single dose, two days, one week or two weeks prior to sacrifice. Nickel ore treatment did not affect lipid peroxidation, protein carbonyl content, aminolevulinic dehydratase, catalase, superoxide dismutase, glutathione peroxidase, glutathione levels, glutathione reductase, glutathione S-transferase (1-nitro-2,4-dichlorobenzene, ethacrynic acid, trans-4-phenyl-3-buten-3-one) in rat liver or kidney to any significant extent. A statistically significant increase was observed in liver NAD(P)H: quinone reductase activity in the group treated with 200 mg/rat (2 w); the

enhancement rate averaged 117%. A significant increase also occurred in kidney glutathione S-transferase (1-chloro-2,4-dinitrobenzene) activity in the groups treated with 100, 200 mg/rat (2 d) and this activation seemed to be induced in a dose-dependent manner. It appears that nickel ore does not pose a serious toxic insult to rats in short term studies and hence the risk posed to aquatic species could be minor. However, long-term studies with rainbow trout and bivalves need to be conducted to investigate possible risks of more broadscale environmental concern.

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## LIST OF ABBREVIATIONS

ALA	aminolevulinic acid
BSA	bovine serum albumin
CAT	catalase
CDNB	1-chloro-2,4-dinitrobenzene
DCIP	2,6-dichlorophenol indophenol
DCNB	1-nitro-2,4-dichlorobenzene
DTT	dithiothreitol
EAA	ethacrynic acid
EDTA	ethylenediamine tetraacetic acid disodium
GR	glutathione reductase
GSH	reduced glutathione
GSH-Px	glutathione peroxidase
GSSG	oxidized glutathione
GST	glutathione S-transferase
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
hr	hour(s)
i.m.	intramuscularly
i.p.	intraperitoneally
KC	kidney cytosol

KH	kidney homogenate
LC	liver cytosol
LH	liver homogenate
LPO	lipid peroxidation
MDA	malondialdehyde
min	minute(s)
NADH	nicotinamide adenine dinucleotide, reduced form
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
OPT	o-phthalaldehyde
Tris	tris (hydroxymethyl) aminomethane
PBG	porphobilinogen
PMAB	p-dimethylaminobenzaldehyde
SOD	superoxide dismutase
TBA	2-thiobarbituric acid
TCA	trichloroacetic acid

## CHAPTER 1

### 1. Introduction

#### 1.1. Identity and solubility of nickel and nickel compounds

Nickel is a silvery white metal belonging to Group VIIIb of the periodic table. Nickel usually has an oxidation state of two while relatively stable tri- and tetra-valent nickel ions occur also. Several binary nickel compounds are commercially and environmentally significant (Stoeppler, 1980). The solubilities of eight Ni compounds in saline solution rank as follows:  $[\text{Ni}(\text{NO}_3)_2 > \text{NiCl}_2 > \text{Ni}_2\text{SO}_4] \gg [\text{NiS} > \text{Ni}_3\text{S}_2] > [\text{NiO (black)} > \text{Ni-metal} > \text{NiO (green)}]$ . The five Ni compounds, NiS,  $\text{Ni}_3\text{S}_2$ , NiO (black), Ni-metal, and NiO (green), which are slightly soluble or insoluble, were assigned to two classes: one is the compounds with solubility less than 5  $\mu\text{g/ml}$ , e.g. NiO (black), Ni-metal, and NiO (green); the other is compounds with the slight solubility of more than 500  $\mu\text{g/ml}$ , e.g. NiS and  $\text{Ni}_3\text{S}_2$  (Ishimtsu *et al.*, 1995).

#### 1.2. Sources of human and environmental exposure

Nickel is a ubiquitous trace metal which occurs in soil, water, air, and in the biosphere. Nickel ore deposits are accumulations of nickel sulfide minerals (mostly pentlandite) and laterite. Nickel is a very widely applied metal. It provides strength and corrosion resistance in alloys. Nickel alloys and nickel platings are applied in vehicles, processing machinery, armaments, tools, electrical equipment, household appliances, and coinage. Nickel compounds are also used as catalysts, pigments, and in batteries. A range of applications may cause human exposures through different pathways. The primary nickel emission into ambient air



comes from combustion of coal and oil for heat or power generation, the incineration of waste and sewage sludge, nickel mining and primary production, steel manufacture, electroplating. Nickel from various sources finally reaches waste water (Hertel *et al.*, 1991).

The following is a list of a number of nickel-exposed occupations identified by the National Institute for Occupational Safety and Health or confirmed by means of biological monitoring: battery makers, ceramic makers, coal gasification workers, dyers, electroformers, electroplaters, enamellers, glass workers, ink makers, jewellers, magnet makers, metal workers, nickel miners, nickel refiners, nickel smelters, oil dehydrogenators, paint makers, sand blasters, spark plug makers, spray painters, stainless steel makers, textile dyes, varnish makers, welders. The absorbed amount of nickel may occasionally exceed 100 µg per day. In the occupational environment, nickel exposure mainly occurs through the respiratory tract. Nickel dusts may enter the body through the gastrointestinal tract due to poor work practices (Grandjean, 1984).

The concentrations of nickel in food are normally below 0.5 mg/kg fresh weight. High concentrations of nickel exist in cocoa, soybeans, some dried legumes, various nuts, and oatmeal. Different dietary habits lead to various daily intake of nickel from food; the mean dietary nickel intake in most countries is 100-300 µg/day. Release of nickel from kitchen utensils and cigarettes smoking may also contribute significantly to nickel intake (Hertel *et al.*, 1991).

### **1.3. Kinetics and metabolism**

#### **1.3.1 Absorption**

Nickel and its inorganic compounds can be absorbed via the gastrointestinal tract or the respiratory passages. The parenteral administration of various nickel compounds is mainly of interest in toxicity studies on animals. Systemic uptake is not necessarily the critical factor, however, because respiratory retention of sparingly soluble nickel is associated with the development of cancer, and cutaneous uptake may lead to an allergic response (Grandjean, 1984). Kuehn & Sunderman (1982) suggested that the *in vitro* dissolution half-times of nickel compounds might be used to predict their *in vivo* elimination half-times, since the dissolution process is rate limiting for the metabolism and elimination of the compounds.

#### **1.3.2 Transport**

Three nickel-binding fractions in blood serum have been identified *in vivo* and *in vitro* studies using labelled nickel chloride:

(a) macroglobulin-bound nickel

(b) albumin-bound nickel

© nickel bound to ultrafiltrable ligands which is mostly in the form of nickel-L-histidine complex.

Exchange and transfer of nickel between L-histidine and albumin appear to be mediated by a ternary complex in the form of albumin-nickel-L-Histidine. It appears that the following equilibria may be present under physiological conditions:

nickel + L-histidine  $\rightarrow$  nickel-L-histidine

nickel-L-histidine + albumin  $\rightarrow$  albumin-nickel-L-histidine

albumin-nickel-L-histidine  $\rightarrow$  albumin-nickel + L-histidine

An intermediate of this nature may make it possible for albumin-nickel to transfer nickel to the low-molecular-weight constituents of human serum, which in turn could transport the metal ion across the biological membrane (Sarkar, 1984). The equilibrium between L-histidine-nickel and serum-albumin-nickel may be biologically significant. The equilibrium in favour of the L-histidine-nickel complex may be an interpretation for the rapid urinary excretion of nickel observed by Onkelinx *et al.* (1973). Under physiological conditions, the relative amount bound to each constituent being dependent on the exact concentration of nickel. The nickel-transport site of human albumin is located at the NH<sub>2</sub>-terminal segment of the protein involving the  $\alpha$ -NH<sub>2</sub> nitrogen, imidazole nitrogen, two deprotonated peptide nitrogens, and the carboxyl side-chain of the aspartyl residue (Sarkar, 1984).

### 1.3.3 Tissue distribution

The reports of the uptake of nickel by organs have differed due to various factors: the nickel compound administered, the route of absorption, the species of animal, the dose, the length of time prior to sacrifice. Several studies have described the kinetics of administered nickel distribution and clearance in experimental animals. Clary (1975) reported the relative distribution of <sup>63</sup>Ni after <sup>63</sup>NiCl<sub>2</sub> (1 mg/kg subcutaneously for 5 days) treatment to Guinea pig as: kidney > pituitary > lung > liver > spleen > heart > adrenal > testis > pancreas > medulla > oblongata = cerebrum = cerebellum. Ishimatsu *et al.* (1995)

reported that, after oral administration of Ni compounds containing 10 mg of Ni, about 84-87% of the Ni was found in the kidneys for the  $\text{NiCl}_2$ ,  $\text{Ni}_2\text{SO}_4$  and NiS groups at 24 hours later. The amount in the kidneys for the  $\text{Ni}_3\text{S}_2$ ,  $\text{Ni}(\text{NO}_3)_2$ ,  $\text{Ni}(\text{NO}_3)_2$ , and Ni-Metal groups was 76, 73, 62, and 51%, respectively. It appears that the highest accumulation of Ni occurred in the kidney, endocrine glands, lung, and liver. The Ni distribution in each organ was related to the solubility of the Ni compound. The Ni distribution in the kidneys increased with increasing solubility. It is clear that the highest concentrations of nickel were found in kidney and urine. It has also been shown that nickel induction of microsomal heme-oxygenase activity is greater in kidney than in any other organs (Sunderman *et al.*, 1983). A nickel-binding protein has been isolated, purified and partially characterized (Sarkar, B., 1984).

#### **1.3.4 Elimination and excretion**

The elimination routes again are determined by the mode of intake and the chemical form of the nickel compound. Urinary excretion is usually the major clearance route for the absorbed nickel. Marina *et al.* (1997) reported the results of the first complete study of nickel metabolism in human subjects using an oral stable nickel isotope ( $^{62}\text{Ni}$ ) as tracer. Evidence of the excretion of absorbed nickel via the gut were not found. The percentage absorption calculated from the amount of  $^{62}\text{Ni}$  excreted in the faeces ranged from 29% to 40%. Urinary excretion over 5 d ranged from 51% to 82% of the absorbed dose. According to Hertel (1991), all body secretions appear to have the ability to excrete nickel, such as saliva, sweat, tears, and milk. Nickel is excreted in the urine, not as the free metal, but bound to a protein that is similar to, or a fragment of, the soluble low relative molecular mass glycoprotein

associated with nickel in renal tissue. Data on nickel excretion suggests a two-compartment model as described by Onkelinx *et al.* (1980).

#### **1.4. Essentiality of nickel**

Kirchgessner and Schnegg (1980) suggested that nickel functions in the body as follows:

- (1) the body can store nickel and has a mechanism to regulate absorption, especially during pregnancy;
- (2) nickel effects the efficiency of iron absorption and consequently blood formation;
- (3) nickel deficiency results in lower activities of many enzymes and in lower substrate and metabolite concentrations;
- (4) nickel deficiency can be induced only by very low nickel contents in the diet;
- (5) the reduced growth rate because of nickel deficiency is preceded by histological and biochemical changes.

Nickel is a component of several enzyme systems (e.g. urease and some hydrogenases) (Costa, 1996). However, definition of specific biochemical functions in higher animals (including humans) for nickel has not been achieved (Nielsen, 1996). The speculation has been presented by Nielsen (1991) that nickel is needed for the CO<sub>2</sub>-fixation to propionyl-CoA to form D-methylmalonyl-CoA in animals and/or humans. Nielsen (1996) suggested that nickel can be included into the “apparent beneficial intake (ABI)” class which denotes the elements with beneficial, if not essential, actions that can be extrapolated from animals to humans.

## **1.5. Nickel toxicity and carcinogenicity**

### **1.5.1. Effects on the respiratory tract**

In human nickel carbonyl poisoning as well as in animals, the lung is the target organ. It was reported that clinical symptoms of acute nickel carbonyl poisoning include both immediate and delayed symptomatology. Pulmonary lesions observed with acute exposure include pulmonary haemorrhage and edema along with deranged alveolar cells, degeneration of bronchial epithelium, and appearance of fibrinous intra-alveolar exudate. In the long term, survivors of the acute episode frequently develop pulmonary fibrosis (Mushak, 1980).

Benson *et al.* (1986) reported the results of single intratracheal doses of nickel subsulfide (3.2, 32, or 320 ug/kg body weight), nickel oxide (3,30, or 300 ug /kg body weight), nickel sulfate (10.5, 105.2, or 1052 ug/kg body weight), and nickel chloride (9.5, 95.2, or 952 ug/kg body weight) on rats. Multifocal alveolitis with some type II hyperplasia was observed in animals treated with nickel chloride, nickel sulfate, or nickel subsulfide at 7 days. In the medium- and high-dose groups of nickel chloride and nickel sulfate and in the highest nickel subsulfide dose group, lung lavage fluid contained increased numbers of neutrophils and macrophages. The relative toxicity ranking was shown as nickel subsulfide > nickel chloride = nickel sulfate > nickel oxide.

Sunderman *et al.* (1989) demonstrated that, in rats treated with single subcutaneous injections of 8-65 mg nickel chloride/kg, alveolar macrophages showed morphological and biochemical signs of activation, functional impairment, and lipid peroxidation.

### **1.5.2 Effects on the endocrine system**

Carbohydrate metabolism may be altered in various animals with nickel challenge. High accumulation of nickel in the pancreas has been found following multiple intraperitoneal injections of nickel acetate. This finding indicated a possible link of nickel with zinc and insulin metabolism which may express as nickel-induced elevation of serum glucose (Hertel *et al.*, 1991).

The hypothalamic tract of animals is also affected by nickel. Dormer *et al.* (1973) showed that the nickel ion is a potent inhibitor of secretion *in vitro* in the parotid gland (amylase), the islets of Langerhans (insulin), and the pituitary gland (growth hormone). The mechanism that nickel may block exocytosis by interfering with either secretory granule migration or membrane fusion and microvilli formation was suggested.

### **1.5.3 Renal effects of nickel**

As summarized by Mushak (1980), exposure of rats to nickel carbonyl resulted in pathological lesions of tubules and glomeruli; aminoaciduria and proteinuria associated with morphological changes were also found in the glomeruli of rats given single injections of nickel chloride were found.

### **1.5.4 Effects on the immune system**

Nickel is known to be a potent allergen in both occupational and nonoccupational exposures. T-cells were reported as a main target population in CBA/J mice given a single i.m. injection of 18.3 mg/kg nickel chloride. Lymphoproliferative response suppression to the B-cell mitogen and natural killer cell activity reduction have been observed due to nickel

chloride exposure. It was also reported that splenocytes from CD rats exposed to nickel sulfide *in vitro* had changed blastogenic responses and cytokine production. It was assumed that the blastogenic properties of Ni may be important in nickel carcinogenicity (Exon *et al.*, 1996).

#### **1.5.5 Mutagenicity of nickel compounds**

Determination of the mutagenic effects of carcinogenic nickel compounds has been difficult because nickel is a poor mutagen or nonmutagenic in prokaryotic mutagenicity assays (Biggart *et al.*, 1987). Nickel compounds are considered to be only weak or equivocal mutagens (Fletcher *et al.*, 1994). Rossetto *et al.* (1994) compared the spontaneous or ethyl methanesulfonate-induced mutants in AS52 (a Chinese hamster ovary cell line) to those generated by exposure to nickel compounds. They observed that nickel compounds exhibited an increase in gene deletions relative to point mutations; the extent of which was compound specific:  $\text{NiSO}_4 > \text{Ni(OH)}_2 > \text{Ni}_3\text{S}_2$ . It was also reported that some nickel compounds were clastogenic *in vitro*, producing chromosome aberrations, transformation and sister chromatid exchanges in mammalian cells (Hertel *et al.*, 1991). In contrast to their weak mutagenicity, nickel compounds have been shown to increase UV-induced cytotoxicity and mutagenicity and to interfere with the repair of UV-induced DNA lesions by disrupting DNA-protein interactions involved in DNA damage recognition (Krueger *et al.*, 1999). Krueger *et al.* (1999) suggested that nickel (II) at non-cytotoxic concentrations inhibits nucleotide excision repair and possibly crosslink repair by interference with distinct steps of the respective repair pathways. Based on the strong correlation between mutagenicity and carcinogenicity,



mutagenicity testing has been suggested to be used as a predictive test for carcinogenicity although it does not give absolutely accurate results (Reith and Brogger, 1984).

#### **1.5.6 Carcinogenicity of nickel and its compounds**

Sunderman (1984) reported that the following incidences of sarcomas occurred at the injection site within two years after male Fischer rats were treated with a single i.m. injection at equivalent dosages (14 mg Ni/rat): nickel subsulfide, 100%; crystalline nickel subsulfide, 100%; nickel ferrosulfide, 100%; nickel oxide, 93%; nickel subselenide, 91%; nickel sulfarsenide, 88%; nickel subselenide, 91%; nickel sulfarsenide, 88%; nickel disulfide, 86%; nickel subarsenide ( $\text{Ni}_3\text{As}_2$ ), 85%; nickel dust, 65%; nickel antimonide, 59%; nickel telluride, 54%; nickel monoselenide, 50%; nickel subarsenide ( $\text{Ni}_{11}\text{As}_3$ ), 50%; amorphous nickel monosulfide, 12%; nickel chromate, 6%; nickel monoarsenide, 0%; nickel titanate, 0%; ferronickel alloy, 0%. Approximately half of the tumors induced were rhabdomyosarcomas; the remainder occurred as fibrosarcomas, undifferentiated sarcomas, unclassified sarcomas, a neurofibrosarcoma, and a fibrous histiocytic sarcoma. Sixty-one percent of the sarcoma-bearing rats had distant metastases. The carcinogenic activities of the compounds were correlated with their nickel mass-fractions, but not with dissolution half-times in rat serum or renal cytosol, or with phagocytic indices by rat peritoneal macrophages *in vitro*.

Ottolenghi *et al.* (1974) reported a high incidence of pulmonary hyperplastic and neoplastic lesions in Fisher 344 rats after exposure to nickel subsulfide (0.97 mg nickel/m<sup>3</sup>) for 6 hr/day, 5 days/week, over 78 weeks.

Lung and nasal cancers have been observed in workers at nickel refineries. The tumors

are dominantly of the epithelial type; anaplastic and pleomorphic tumors are seldom found. The information for other occupation exposure such as welding, electroplating, grinding, work with nickel catalysts is less extensive than for nickel refining. Cancer on sites of the larynx, of the stomach and of the kidney, and sarcomas of soft tissues have also been attributed to nickel exposure (Leonard, 1981).

## **1.6. The possible mechanism involved in nickel toxicity and carcinogenicity**

### **1.6.1 Carcinogenicity mechanistic model of nickel compounds**

The carcinogenic potencies of different nickel compounds appear to be related to the bioavailability of nickel (presumably  $\text{Ni}^{2+}$ ) to critical intracellular sites (Costa, 1991). According to Oller (1997), it is recognized that there are two main components that could contribute to the development of lung cancer due to nickel exposure:

#### **I. The genetic or epigenetic heritable changes derived from the actions of nickel compounds**

The heritable changes can be attributed to two classes:

- (a) direct effects (nickel specific): the changes which result from a direct  $\text{Ni}^{2+}$  effect on DNA/chromosomes (e.g. nickel subsulfide)
- (b) indirect effects (not nickel specific): the changes which can be indirectly induced by nickel compounds as a consequence of an inflammatory response, such as DNA damage caused by oxygen radicals (e.g. nickel oxide)

#### **II. The promotion of cell proliferation elicited by certain nickel compounds (not nickel specific, e.g. nickel sulphate).**

Both components are required to produce cancer. During the occurrence of direct effects,

three steps are involved: (a) the nickel compound particles are endocytized by the target cells; (b) the endocytic vesicles are acidified by fusion with lysosomes and  $\text{Ni}^{2+}$  is released; (c) the endocytic vesicles merge with the nuclear membrane delivering  $\text{Ni}^{2+}$  to the nucleus, particularly to the heterochromatic region of the DNA located in close proximity to the inner nuclear membrane. The following events may ensue: condensation and methylation enhancement of the DNA nearby region which could result in heritable inactivation of tumor suppressor genes; generation of oxygen radicals through a  $\text{Ni(III)/Ni(II)}$  mechanism which subsequently induces damaged bases, DNA strand breaks, and DNA-protein crosslinks, chromosomal aberrations.

### **1.6.2 Nickel induced oxidative stress and antioxidant defence perturbation**

#### **1.6.2.1 Introduction of oxidative stress and antioxidant defence system**

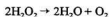
The term oxidative stress is widely used in the free-radical literature but it is rarely defined. In essence, it refers to the situation of a serious imbalance between production of ROS (reactive oxygen species)/RNS (reactive nitrogen species) and antioxidant defence (Halliwell and Gutteridge, 1999).

##### **1.6.2.1.1 Lipid peroxidation and related defence enzymes**

One of the mechanisms of damage to cellular targets by oxidative stress is lipid peroxidation. Lipid peroxidation has been defined as “the oxidative deterioration of polyunsaturated lipids”. Membranes in eukaryotes contain many essential polyunsaturated fatty acids (PUFA). Initiation of lipid peroxidation is caused by attack upon an unsaturated lipid of any species that has sufficient reactivity to abstract a hydrogen atom from a methylene

(-CH<sub>2</sub>-) group. Once the free-radical chain reaction is initiated, it propagates until the unsaturated lipid is exhausted or two free radicals destroy each other to terminate the chain (Halliwell and Gutteridge, 1999). The products of lipid peroxidation include highly reactive lipid hydroperoxides, H<sub>2</sub>O<sub>2</sub>, hydroxyl radical, and malondialdehyde that may produce genotoxic effects, e.g. DNA degradation, DNA strand breaks, protein-DNA cross links, and oxidative modification of DNA bases (Misra, 1991).

In biological systems, there exist enzymes which are capable of controlling the cytotoxic effects of active oxygen species, including **catalase (CAT)**, **glutathione peroxidase (GSH-Px)** and **superoxide dismutase (SOD)** which can protect cells from the accumulation of H<sub>2</sub>O<sub>2</sub> hydroperoxides, and superoxide (Misra *et al.*, 1990). The advent of oxidative stress *in vivo* often leads to increased levels of antioxidant enzymes which serve to protect the organism/tissue from the deleterious effects of reactive oxygen species and lipid peroxidation products (Barros *et al.*, 1991). The reaction catalysed by CAT is the following (Aebi *et al.*, 1984):



**NAD(P)H: quinone oxidoreductase**, also named **DT diaphorase**, which is often regarded as a phase II detoxification system, catalyses a two-electron reduction of quinones into hydroquinones at the expense of NADH or NADPH. One function of quinone reductase is to diminish the formation of superoxide radicals *in vivo* by removing quinones. Moreover, quinone reductase has been implicated to help maintain coenzyme Q in the reduced form, hence “recycling” it as an inhibitor of lipid peroxidation (Halliwell & Gutteridge, 1999).

#### 1.6.2.1.2 Protein carbonyls

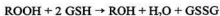
Metal-catalyzed oxidation has been identified as a post-translational covalent modification of proteins which may be important in several physiological and pathological processes which include the aging process, intracellular protein turnover, arthritis, and pulmonary diseases. Introduction of carbonyl groups into amino acid residues of proteins is a hall mark for oxidative modification (Levine, 1990).

The carbonyl assay is a general assay of oxidative protein damage. It is based on the fact that several ROS attack amino-acid residues in proteins to produce products with carbonyl groups (Halliwell and Gutterage, 1999).

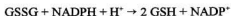
#### 1.6.2.1.3 Glutathione and its metabolising enzymes

Glutathione (GSH) is a nonprotein thiol tripeptide, which is present in high concentrations in all cells including those of liver and kidney. It has several important functions e.g. the maintenance of sulfhydryl enzymes, integrity of membranes against peroxidative damage, regulation of amino acid transport and maintenance of intracellular redox potential (Athar *et al.*, 1987). Moreover, glutathione is recognised as a protective compound within the body for the removal of xenobiotics by forming soluble conjugates and facilitating their excretion from the cell. The enzymes catalysing the above conjugations are **glutathione-S-transferases (GST)** which are located in the cytosol of liver, kidney, gut and other tissues (Gibson, 1994). **Glutathione peroxidase**, an enzyme that reduces  $H_2O_2$  and lipid peroxidation like catalase at the expense of GSH, is known as a major protective system against endogenously and exogenously induced lipid peroxidation. The reaction it catalyses is as follows (Wendel,

1981):



**Glutathione reductase** regenerates GSH at the expense of NADPH which plays an important role in maintaining a high ratio of reduced to oxidized glutathione (GSH/GSSG) in normal cells. The reaction catalysed is as follows (Halliwell and Gutteridge, 1999):



#### **1.6.2.1.4 Heme metabolizing system**

Heme constitutes the prosthetic moiety of a variety of hemoproteins. The list includes the oxygen binding proteins hemoglobin and myoglobin, catalases and a large number of peroxidases and all the cytochromes P450. Thus, an alteration in the heme content of the cell could profoundly effect the activity of several cellular enzymes and therefore, cellular functions. The diminution of cellular heme content by the combination of inhibiting the heme biosynthetic pathway (e.g. **aminolevulinic acid dehydratase** ) and/or stimulating the heme degradation pathway (e.g. **heme oxygenase**) by metal ions could have a significant impact on the cell. (Maines, 1980).

#### **1.6.2.2 Nickel induced oxidative stress and antioxidant defense system perturbation**

Extensive studies have been conducted in view of the fact that nickel induces oxidative stress and perturbs antioxidant defence systems. Based on animal studies, lipid peroxidation in both liver and kidney could be induced with nickel exposure (Misra *et al.*, 1991; Athar *et al.*, 1987; Srivastava *et al.*, 1990; Misra *et al.*, 1990). Nickel can also diminish heme levels through the combination of heme oxygenase activity inhibition and ALA dehydratase activity

enhancement (Trevisan *et al.*, 1980; Sunderman *et al.*, 1983; Iscan *et al.*, 1992; Maines, 1980). GSH levels and the activity of antioxidant enzymes such as GST, GR, GSH-Px, SOD and CAT have also been shown to be affected by nickel in a strain- and time-dependent manner concurrently with lipid peroxidation enhancement (Herrero *et al.*, 1993; Cartana *et al.*, 1989; Athar *et al.*, 1987<sup>a</sup>; Misra *et al.*, 1991; Srivastava *et al.*, 1990; Athar *et al.*, 1987<sup>b</sup>; Misra *et al.*, 1990; Rodriguez *et al.*, 1990; Iscan *et al.*, 1992, 1993; Cartana *et al.*, 1992;).

### **1.7. Objective of the thesis**

There is increasing concern over the possibility of toxic metals leaching from mine tailings and from the mines themselves. A recent study from a Ni mine and smelter site in Norway indicated enhanced levels of Ni, Co, Cu and Zn in the land surrounding the smelter, in water draining from the mine and smelter and in fish (brown trout) caught from sites immediately downstream. Nickel is known to accumulate in the gills, liver, kidney, brain and white muscle of fish (Brotheridge *et al.*, 1998).

This project will involve sub-lethal toxicity studies associated with byproducts of the mining and smelting of the nickel rich pentlandite (Ni, Fe, Co)<sub>9</sub>S<sub>8</sub> from Voisey's Bay. The Voisey's Bay nickel deposit is located near the coastal village of Voisey's Bay in Labrador Newfoundland, Canada. Voisey's Bay is deemed to be the most significant mineral discovery in Canada in 30 years and disposal of the 4 million tons of tailings (containing Ni, Cu, Co and Fe) to be produced annually is recognized by DFO (Department of Fisheries and Oceans) and Environment Canada to be a major issue. The tailings, which have the potential to generate acid in the presence of oxygen, are to be pumped by pipeline into a number of ponds which

will decant to some extent to the ocean. It is proposed to use aqueous suspensions of nickel ore and nickel subsulfide to assess their uptake and toxicity potentials in a variety of systems. Initially, *in vivo* studies using rats will be carried out. Information obtained from these studies will subsequently be used to design long-term studies with rainbow trout and bivalves. Overall, this study aims to determine if the leachable as well as particulate lattice forms of the metals are taken up to any extent and pose risks of more broadscale environmental concern. It is worth noting that the studies with nickel ore would involve a mixture of metals and the response of organisms to it may be quite different from the response to pure metal compounds used in most studies.

My research is the first step of the project aiming to establish the toxic response of rats to nickel ore with respect to oxidative damage, antioxidant and detoxicating enzymes. With regard to carcinogenicity assessment, nickel subsulfide has been suggested as the Ni compound which presents the highest carcinogenic potential relative to other Ni compounds (Oller *et al.*, 1997). The carcinogenic potency of all Ni compounds is directly related to their ability to enter cells, which is correlated with compound solubility. It is postulated that the manner in which nickel is complexed in the ore and its solubility (or lack of it) will have a significant impact on how it affects organisms. In this regard, the effects of nickel ore may resemble that of nickel subsulfide since the latter is also insoluble. Therefore, the response of rats to both nickel subsulfide and nickel ore was investigated in my project. The following biochemical parameters were examined:

(1) ALA dehydratase



- (2) antioxidant system which contains: catalase, SOD, glutathione peroxidase
- (3) glutathione level
- (4) glutathione S-transferase
- (5) glutathione reductase
- (6) NAD(P)H: quinone reductase
- (7) lipid peroxidation level
- (8) protein carbonyl content

## CHAPTER 2

### 2. MATERIALS AND METHODS

#### 2.1 Materials

##### 2.1.1 Chemicals

DTT, EDTA, HEPES, protease inhibitor cocktail, TBA, GSH, OPT, dinitrophenylhydrazine, ethyl acetate, ALA hydrochloride, PMAB,  $\text{HgCl}_2$ , succinic anhydride, ferricytochrome c, hypoxanthine, xanthine oxidase, sodium azide, glutathione reductase, NADPH, NADH, GSSG, CDNB, DCNB, ethacrynic acid, trans-4-phenyl-3-buten-2-one, FAD and DCIP were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Nickel ore was from PCMR CANMET (Ni, 1.233 wt%; Cu, 0.967 wt%; Co, 0.041 wt%; Pt, 0.41 ug/g; Pd, 0.37 ug/g; Ag, 4.30 ug/g ). Nickel subsulfide (  $\text{Ni}_3\text{S}_2$  ) was purchased from Inco Technical Services Limited (Mississauga, Ontario, Canada). All other chemicals were of the highest grade commercially available.

#### 2.2 Methods

##### 2.2.1 Animal treatment

Male Sprague-Dawley rats (165~175 g) were obtained from Animal Care Services, Memorial University of Newfoundland, and maintained on standard laboratory rat chow and water ad lib. Nickel subsulfide or Nickel ore was administered intraperitoneally (i.p.) as a suspension in 1:1 glycerol and saline (0.9% NaCl). The various treatments are listed below:

- 1) Nickel subsulfide (100 mg/rat ), i.p. , as a single dose, sacrificed in 2 weeks,
- 2) Nickel subsulfide (200 mg/rat), i.p., as a single dose, sacrificed in 2 weeks,

- 3) Nickel subsulfide (200 mg/rat), i.p., daily for 2 days, sacrificed in 2 weeks,
- 4) Nickel ore (100 mg/rat), i.p., as a single dose, sacrificed in 2 weeks,
- 5) Nickel ore (200 mg/rat), i.p., as a single dose, sacrificed in 2 weeks,
- 6) Nickel ore (200 mg/rat), i.p., as a single dose, sacrificed in 1 week,
- 7) Nickel ore (100 mg/rat), i.p., as a single dose, sacrificed in 2 days,
- 8) Nickel ore (200 mg/rat), i.p., as a single dose, sacrificed in 2 days.

Control rats received an equivalent amount of vehicle (1:1 glycerol-saline). Rats were fasted for 12 hours prior to being killed by cervical dislocation. Livers were perfused *in situ* with 0.9% NaCl.

## **2.2.2 Preparation of samples**

### **2.2.2.1 Preparation of liver cytosol and the samples for measurement of GSH, lipid peroxidation and ALA dehydratase activity**

All the following procedures for the preparation of **liver cytosol** and **kidney cytosol** were performed at 0-4°C according to the method described by Rahimtula *et al.* (1979). A piece of each liver (around 0.3 g) was taken immediately after perfusion and frozen rapidly in liquid nitrogen for the assay of **glutathione content measurement** (Akerbroom, 1981). The frozen liver samples then were stored at -70°C before use. Another piece of each liver around 0.3 g was put individually into the specific buffer which will be described in section 2.2.2.2 for **protein carbonyl assay**. The rest of the liver was washed in saline. After weighing, the liver was minced into fine pieces and homogenized in 3 volumes (w/v) of 0.1 M potassium phosphate buffer (pH 7.4) by a motor-driven Potter-Elvehjem homogenizer.

One ml of the homogenate was taken, mixed with EDTA (final concentration 1 mM), and stored (at -70°C before use) for **lipid peroxidation measurement** using the MDA-TBA method. The rest of the homogenate was centrifuged at 2,000 g for 5 min, then at 10,000 g for 10 min.

Liver cytosol was isolated from the 10,000 g supernatant by centrifugation at 105,000 g for 75 min. An aliquot of 0.83 ml of each liver cytosol was taken and individually mixed with 0.02 ml of 250 mM DTT (dithiothreitol) and 0.15 ml glycerol. The mixture was then frozen in liquid nitrogen and stored at -70°C before use for the measurement of **ALA dehydratase activity** (Sassa, 1982).

#### **2.2.2.2 Preparation of samples for protein carbonyl content measurement**

The liver sample taken in the section 2.2.2.1 was homogenized in 3 volumes (w/v) of buffer which contained HEPES (10 mmol/L, pH 7.4), NaCl (137 mmol/L), KCl (4.6 mmol/L),  $\text{KH}_2\text{PO}_4$  (1.1 mmol/L), magnesium sulphate (0.6 mmol/L), and ethylenediaminetetraacetic acid (1.1mmol/L). Protease inhibitor cocktail (16.66 ml/ L) was also added to inhibit proteases in the samples. The liver homogenate was centrifuged (4 °C) first at 10,000 g for 15 min. The supernatant used for the determination of protein carbonyl content was recovered after centrifugation of the 10,000 supernatant at 105,000 g for 5 min at 4°C. The samples were kept at -70°C before use (Rouach *et al.*, 1997).

#### **2.2.2.3 Preparation of kidney cytosol**

The kidneys from each rat were excised and pooled separately in ice-cold 0.1M potassium phosphate buffer (PH 7.4). The kidneys were then sliced in half and the cortex was

carefully cut off from the medulla with scissors. The renal cortex was minced into fine pieces and homogenized in 3 volumes (w/v) of 0.1M potassium phosphate buffer (PH 7.4) by a motor-driven Potter-Elvehjem homogenizer (2 X 15 sec at a setting of 5). In some cases, 1 ml of the homogenate was taken, mixed with EDTA (final concentration 1 mM), and stored at -70°C before the measurement of lipid peroxidation. The rest of the homogenate was centrifuged first at 2,000 g for 5 min, then at 10,000 g for 10 min. Kidney cytosol was isolated from the 10,000 g supernatant by centrifugation at 105,000 g for 75 min and stored at -70°C before use.

#### **2.2.2.4 Preparation of succinoylated cytochrome c**

Partially succinoylated cytochrome c was made according to Kuthan *et al.* (1982). Finely grained succinic anhydride (0.42 mmol, 42 mg ) was added to a vigorously stirring solution of ferricytochrome c (8  $\mu$ mol, 100 mg) in 40 ml of ice-cold 30 mM potassium phosphate buffer (pH 7.6) over a period of 30 min. The pH of the solution was kept at 7.6 by titration with 2 M KOH. After the reaction is complete, the solution was stirred for a further 20 min and then transferred to a dialysis bag and dialyzed overnight against doubly distilled water containing 0.1 mM EDTA at 4°C. The modified cytochrome c preparation was concentrated about 3-fold by ultrafiltration through PM 10 membrane and stored at -20°C.

#### **2.2.3 Protein determination**

The protein concentration of all samples was determined by the method of Lowry *et al.* (1951).

#### **2.2.4 Measurement of lipid peroxidation**

Lipid peroxidation in the samples was monitored by measuring the level of MDA as described by Uchiyama and Mihara (1978). In a 10 ml test tube, 0.5 ml (5 mg protein) of homogenate was mixed with 3 ml of 1%  $H_3PO_4$  to keep the pH of the medium at about 2.0. One ml of 0.6% aqueous TBA was then added and the mixture was heated at 100°C for 45 min. After cooling, 4 ml of 1-butanol was added and the mixture was shaken vigorously. The butanol phase was separated by centrifugation (2,500 g for 10 min) and its absorbance at 535 and 520 nm was measured. The difference in absorbance at these two wavelengths was taken to avoid interference due to the protein. Tetramethoxypropane was used as an external standard to calculate the MDA-TBA value as MDA is released by the hydrolysis of tetramethoxypropane. The level of MDA-TBA is expressed as nanomole of MDA per mg protein.

#### **2.2.5 Measurement of GSH content in liver homogenate**

GSH content in liver homogenate was measured according to Hissin and Hilf (1976). A portion of 250 mg liver tissue was homogenized on ice using a Polytron homogenizer. The solution used for homogenization consisted of 3.75 ml of 0.1M sodium phosphate buffer (pH 8.0) containing 0.005 M EDTA and 1 ml of 25% metaphosphoric acid ( $HPO_3$ ). The total homogenate was centrifuged (4°C) at 105,000 g for 30 min to obtain the supernatant for the assay of GSH. An 0.5 ml aliquot of the supernatant was mixed with 4.5 ml 0.1M sodium phosphate buffer (pH 8.0) containing 0.005 M EDTA. The final assay mixture (2.0 ml) contained 100 µl of the diluted tissue supernatant, 1.8 ml of phosphate-EDTA buffer, and

100  $\mu$ l of the OPT (o-phthalaldehyde) solution, containing 100  $\mu$ g of OPT. After thorough mixing and incubation at room temperature for 15 min, the solution was transferred to a quartz cuvette. Fluorescence at 420 nm (slit 10) was determined with activation at 350 nm (slit 5) (scale is fixed at 1.0). A standard curve was constructed by using known amounts of GSH instead of diluted tissue homogenate (0, 0.1, 0.5, 1.0, 1.5, 2.0  $\mu$ g GSH per 2 ml reaction mixture).

#### **2.2.6 Measurement of protein carbonyl content in liver homogenate**

The determination of protein carbonyl content was conducted according to Levine *et al.* (1990). An aliquot of 1 mg protein in 0.15 ml 0.1M potassium phosphate buffer (pH 7.4) was mixed with 0.6 ml of 10 mM 2,4-dinitrophenylhydrazine in 2 M HCl. The blank was run by adding 2 M HCl instead of 2,4-dinitrophenylhydrazine. The mixture was allowed to stand at room temperature for 1 hour, with vortexing every 10-15 min. Then 0.75 ml 20% TCA was added. The mixture was centrifuged at 11,000 g for 3 min and the supernatant discarded. The pellet was washed 3 times with 1.5 ml ethanol-ethyl acetate (1:1) to remove free reagent, allowing the sample to stand 10 min before each centrifugation. The precipitated protein was redissolved in 0.9 ml of 6 M guanidine in 20 mM potassium phosphate (adjusted to pH 2.3 with trifluoroacetic acid) at 37°C for 15 min. Any insoluble material was removed by centrifugation for 3 min at 11,000 g. A spectrum of the sample in the wavelength range of 360–390 nm was obtained with the complementary blank sitting at the reference position. The carbonyl content was calculated from the maximum absorbance using a molar absorption coefficient of 22,000 M<sup>-1</sup> cm<sup>-1</sup>.

## **2.2.7 Measurement of enzyme activities**

### **2.2.7.1 delta-Aminolevulinic acid dehydratase**

The measurement of delta-aminolevulinic acid dehydratase was carried out following the method described by Sassa (1982). The assay stock mixture was made as follows: 0.8 ml of 100 mM ALA, 0.2 ml of 1 M DTT and 8.0 ml of 50 mM sodium phosphate buffer (pH 5.8) were mixed and adjusted to pH 5.8. Then the volume of the solution is brought up to 10 ml with the same buffer. The modified Ehrlich's reagent was made as follows: 0.7 g  $\text{HgCl}_2$  was dissolved in 168 ml acetic acid (warmed gently). After cooling, 40 ml of 70%  $\text{HClO}_4$  was added and the mixture was diluted to 220 ml with acetic acid. One gram of p-dimethylaminobenzaldehyde (PMAB) was dissolved in 55 ml of the above solution prior to use. The modified Ehrlich's reagent was kept in a brown bottle because it is light sensitive.

The assay mixture contained 0.5 ml of the above assay stock mixture or assay stock mixture devoid of ALA hydrochloride and 50  $\mu\text{l}$  of the sample prepared by the procedure mentioned under sample preparation section 2.2.2.1. The mixture was incubated at 37°C for 60 min in the dark. One and a half ml of 6% TCA solution containing 0.1M  $\text{HgCl}_2$  was added to stop the reaction. After centrifugation at 1,000 g for 5 min, an aliquot of 1 ml supernatant was mixed with 1 ml Ehrlich's reagent. After exactly 10 min at 23 °C when the maximal absorbance of the Ehrlich -PBG(porphobilinogen) colour salt developed, the absorbance was measured at 553 nm (extinction coefficient  $6.1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ ) .

### **2.2.7.2 Catalase**

Catalase in liver and kidney cytosol was measured according to Aebi, 1984. Catalase



activity was estimated by measuring the absorbance decrease of  $\text{H}_2\text{O}_2$  at 240 nm (20 °C) . The assay was carried out in a 3 ml cuvette containing 50 mM potassium phosphate buffer (pH 7.0) and an appropriate amount of protein (0.005 mg liver cytosol protein or 0.01 mg kidney cytosol protein). The reaction was started by addition of the cytosol (mixed well with a plastic paddle) . The absorbance decrease was recorded for about 1 min. The molar extinction coefficient of  $\text{H}_2\text{O}_2$  is  $43.6 \text{ M}^{-1} \text{ cm}^{-1}$ .

#### **2.2.7.3 Superoxide dismutase (SOD)**

Enzyme activity was measured by a modification of the method described by Flohe and Otting (1984). Solution A was prepared as follows: 5  $\mu\text{mol}$  (0.68 mg) hypoxanthine in 10 ml 0.001 N sodium hydroxide and 2  $\mu\text{mol}$  modified cytochrome c (see part 2.2.2.4) were admixed with 100 ml of 50 mM phosphate buffer (pH 7.8) containing 0.1 mM EDTA. Solution B was a freshly prepared solution of xanthine oxidase in 0.1 mM EDTA (0.2 unit/ml). The assay was carried out at 25 °C in a 3 ml spectrophotometer cuvette. Solution A (2.9 ml) was pipetted into the cuvette followed by 50  $\mu\text{l}$  of sample and 10  $\mu\text{l}$  of 3 mM sodium azide (final concentration  $10^{-5} \text{ M}$ ; to block peroxidases). The reaction was started with 50  $\mu\text{l}$  of solution B (xanthine oxidase). After mixing, the absorbance change at 550 nm was recorded. Under these defined conditions, the amount of SOD (cytosol) required to inhibit the rate of reduction of cytochrome C by 50% is defined as 1 unit of activity.

#### **2.2.7.4 Glutathione peroxidase**

Glutathione peroxidase activity was measured as described by Flohe and Gunzler

(1984). The following solutions are pipetted into a 1 ml cuvette: five hundred  $\mu\text{l}$  0.1 M phosphate buffer (pH 7.0), 100  $\mu\text{l}$  enzyme sample (0.02 mg liver cytosol protein or 0.04 mg kidney cytosol protein), 100  $\mu\text{l}$  glutathione reductase (0.24 unit), and exactly 100  $\mu\text{l}$  of 10 mM GSH . One milimolar sodium azide was added to block catalase in the sample. The mixture was preincubated for 10 min at 37°C. Thereafter, 100  $\mu\text{l}$  1.5 mM NADPH in 0.1%  $\text{NaHCO}_3$  was added and hydroperoxide-independent consumption of NADPH was monitored for 3 min. The overall reaction was started by adding 100  $\mu\text{l}$  of prewarmed 1.5 mM hydrogen peroxide solution and the decrease in absorbance at 340 nm was monitored for about 5 min. The nonenzymic reaction rate was correspondingly assessed by replacing the enzyme sample by buffer. The activity of the enzyme was calculated as follows:

$$A = 0.868 (\text{change of } [\text{NADPH}]/[\text{GSH}]_0 t) (V_i/V_e)$$

Note:  $[\text{GSH}]_0$  : initial concentration of GSH

Change of  $[\text{NADPH}] / t$  : the rate of decrease in NADPH concentration

$V_i$  : the volumes of the incubation mixture

$V_e$  : the volumes of the enzyme sample

The extinction coefficient of NADPH at 340 nm is  $6.22 \text{ mM}^{-1} \text{ cm}^{-1}$  .

### **2.2.7.5 Glutathione reductase**

The assay was carried out in 3.0 ml cuvettes at 25 °C and contained (final concentrations): 50 mM potassium phosphate buffer (pH 7.0), 0.2 M KCl, 1 mM EDTA, 1 mM GSSG, cytosolic protein (liver cytosol protein 0.4 mg or kidney cytosol protein 0.2 mg ) and 0.1 mM NADPH . The rate of the reaction was followed by recording the decrease in

absorbance of NADPH at 340 nm as GSSG was reduced to GSH (Worthington and Rosemeyer, 1974).

#### **2.2.7.6 Glutathione S-transferases**

Glutathione S-transferase activity was measured as described by Habig *et al.* (1974) using CDNB (1-chloro-2,4-dinitrobenzene), DCNB (1-nitro-2,4-dichlorobenzene), ethacrynic acid and trans-4-phenyl-3-buten-2-one as acceptor substrates. All reactions were carried out at 30°C in 3 ml spectrophotometer cuvettes and were initiated by the addition of GSH. A complete assay mixture without enzyme served as the control. All the substrate solutions were prepared with ethanol as solvent.

##### **2.2.7.6.1 CDNB as substrate**

The 3 ml mixture contained 0.1 M potassium phosphate buffer (pH 6.5), 1 mM CDNB, 0.005 mg liver cytosol protein or 0.04 mg kidney cytosol protein and 1 mM GSH. The reaction was followed by measuring the change in absorbance at 340 nm. The millimolar extinction coefficient is  $9.6 \text{ mM}^{-1} \text{ cm}^{-1}$ .

##### **2.2.7.6.2 DCNB as substrate**

The 3 ml mixture contained 0.1 M potassium phosphate buffer (pH 7.5), 1 mM DCNB, 0.1 mg liver cytosol protein or 4 mg kidney cytosol protein and 5 mM GSH. The reaction was followed by measuring the change in absorbance at 345 nm. The millimolar extinction coefficient is  $8.5 \text{ mM}^{-1} \text{ cm}^{-1}$ .

##### **2.2.7.6.3 Ethacrynic acid**

The 3 ml mixture contained 0.1 M potassium phosphate buffer (pH 6.5), 0.2 mM

ethacrynic acid, 0.4 mg liver cytosol protein or 1 mg kidney cytosol protein and 0.25 mM GSH. The reaction was followed by measuring the change in absorbance at 270 nm. The milimolar extinction coefficient is  $5.0 \text{ mM}^{-1} \text{ cm}^{-1}$ .

#### **2.2.7.6.4 trans-4-Phenyl-3-buten-2-one as substrate**

The 3 ml mixture contained 0.1 M potassium phosphate buffer (pH 6.5) , 50  $\mu\text{M}$  trans-4-phenyl-3-buten-2-one, 0.4 mg liver cytosol protein or 3 mg kidney cytosol protein and 0.25 mM GSH . The reaction was followed by measuring the change in absorbance at 290 nm. The milimolar extinction coefficient is  $24.8 \text{ mM}^{-1} \text{ cm}^{-1}$ .

#### **2.2.7.7 NAD(P)H: quinone reductase**

The assay was carried out as described by Benson *et al.* (1980). The reaction mixture contained in a final volume of 3.0 ml : 25 mM Tris-HCl (pH 7.4), 0.7 mg bovine serum albumin (BSA) at pH 7.4, 0.01% by volume Tween 20, 5  $\mu\text{M}$  FAD, 0.2 mM NADH, 0 or 10  $\mu\text{M}$  dicoumarol, an appropriate amount of cytosol (0.1 mg liver cytosol protein or 0.2 mg kidney cytosol protein), and 40  $\mu\text{M}$  dichloroindophenol (DCIP). The assay was conducted at 25 °C with and without dicoumarol. The dicoumarol-sensitive part of the activity was taken as a measure of the quinone reductase activity. The initial velocity of the reduction of DCIP was measured spectrophotometrically at 600 nm using an extinction coefficient of  $2.1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ .

## C H P T E R 3

### 3. RESULTS

Rats in the nickel subsulfide treated group generally showed very slow weight gain compared with the control group. Fluid in the abdomen was found in one rat in the group treated with 200 mg nickel subsulfide (2 doses, 2 w); another rat in the same group appeared very sick. Rats treated with 100 mg nickel ore (1 dose, 2 w) (II) had significant slower weight gain (with  $50.43 \pm 5.49$  percent compared with  $56.64 \pm 3.30$  percent in control group). Weight gain in rats treated with 200 mg nickel ore (1 dose, 2 w) (III) showed no significant difference compared with the control group. Overall, Ni ore treatment appears to exert no effect on rat weight gain.

#### **3.1 *In vivo* effect of nickel ore on LPO levels in rat liver and kidney homogenate**

MDA levels were measured in liver and kidney homogenates to observe the effect of nickel ore injection upon lipid oxidation. As shown in Table 3.1, a significant increase in liver lipid peroxidation by nickel ore was observed only in one of the three groups treated with nickel ore 200 mg/rat, i.p., as one single dose and sacrificed in 2 weeks. No significant changes in lipid peroxidation levels were observed in the other two repeated trials. In fact, a significant decrease in liver lipid peroxidation levels was observed in the group treated with nickel ore 200 mg/rat, i.p., as one single dose, sacrificed in 2 days. The other treatments of 100 mg or 200 mg nickel ore showed a trend towards lower lipid peroxidation levels in both kidney and liver although the differences were not significant. Overall, it appears that Ni ore does not increase MDA levels in the liver or kidney of treated rats.

**Table 3.1**

The effect of nickel ore on lipid peroxidation in rat liver and kidney homogenate (pmol/mg protein)

	Ni <sub>3</sub> S <sub>2</sub> 100 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 doses 2 w	Ni ore 100 mg/rat 2 w (I)	Ni ore 100 mg/rat 2 w (II)	Ni ore 200 mg/rat 1 w	Ni ore 200 mg/rat 2 w (I)	Ni ore 200 mg/rat 2 w (II)	Ni ore 200 mg/rat 2 w (III)	Ni ore 100 mg/rat 2 d	Ni ore 200 mg/rat 2 d
LH control	not done	not done	not done	not done	201.15 ± 63.90	173.00 ± 26.00	not done	174.77 ± 39.91	201.15 ± 63.90	277.65 ± 23.85	277.65 ± 23.85
treated					138.60 ± 64.80	245.00 ± 110.00		236.86 ± 31.67**	142.2 ± 39.60	241.65 ± 40.95	220.5 ± 48.15*
KH control	not done	not done	not done	not done	649.35 ± 229.50	not done	not done	not done	649.35 ± 229.50	571.05 ± 40.95	571.05 ± 40.95
treated					512.10 ± 87.30				542.25 ± 52.65	405.45 ± 93.15	415.35 ± 85.05

Values shown are means ± S.D. of duplicate determinations from 6 individual rats per nickel ore treatment group

\* denotes a significant difference ( $P < 0.05$ , t test)

\*\* denotes a significant difference ( $P < 0.01$ , t test)

\*\*\* denotes a significant difference ( $P < 0.001$ , t test)

LH: liver homogenate

KH: kidney homogenate

### **3.2 *In vivo* effect of nickel ore upon liver homogenate protein carbonyl content**

The reports investigating the effect of nickel on hepatic protein carbonyl content have not been found in a review of the literature. Protein carbonyl content in rat liver was measured in particular to assess the effect of nickel ore on oxidative protein damage concurrently with lipid peroxidation. As shown in Table 3.2, no significant changes in liver protein carbonyl content were observed for all the treatments of nickel ore compared with controls. These results are in keeping with the lack of lipid peroxidation changes mentioned in part 3.1. It appears that nickel ore treatment does not enhance of the oxidation of lipids and proteins.

**Table 3.2**

The effect of nickel ore on protein carbonyl content in rat liver (pmol/mg protein)

	Ni <sub>3</sub> S <sub>2</sub> 100 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 doses 2 w	Ni ore 100 mg/rat 2 w (I)	Ni ore 100 mg/rat 2 w (II)	Ni ore 200 mg/rat 1 w	Ni ore 200 mg/rat 2 w (I)	Ni ore 200 mg/rat 2 w (II)	Ni ore 200 mg/rat 2 w (III)	Ni ore 100 mg/rat 2 d	Ni ore 200 mg/rat 2 d
control	not done	not done	not done	not done	647.20 ± 222.73	not done	not done	507.08± 133.06	647.20 ± 222.73	589.79 ± 96.52	589.79 ± 96.52
treated					490.93 ± 143.48			531.31± 46.00	687.29 ± 175.29	498.29 ± 119.67	602.33 ± 186.23
control	not done	not done	not done	not done	not done	not done	not done	not done	not done	not done	not done
treated											

Values shown are means ± S.D. of duplicate determinations from 6 individual rats per nickel ore treatment group

- \* denotes a significant difference (P < 0.05, t test)
- \*\* denotes a significant difference (P < 0.01, t test)
- \*\*\* denotes a significant difference (P < 0.001, t test)



### **3.3 *In vivo* effect of nickel ore upon ALA dehydratase activity in rat liver**

The activity of ALA dehydratase is known to be inhibited in several tissues including liver and kidney by administration of nickel chloride to rats (Maines, 1977). Although ALA dehydratase is not regarded as the regulatory enzyme in the heme biosynthesis pathway (Maines, 1980), the alteration of this enzyme activity will indicate heme biosynthesis perturbation. However, an insoluble form of nickel (e.g. the form in nickel ore) may act rather differently from the soluble one. As shown in Table 3.3, no significant changes in rat liver ALA dehydratase activity were observed for all the treatments of nickel ore compared with control group.

**Table 3.3**

The effect of nickel ore on ALA dehydratase activity in rat liver and kidney (nmol PBG/hour/mg protein)

	Ni <sub>3</sub> S <sub>2</sub> 100 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 doses 2 w	Ni ore 100 mg/rat 2 w (I)	Ni ore 100 mg/rat 2 w (II)	Ni ore 200 mg/rat 1 w	Ni ore 200 mg/rat 2 w (I)	Ni ore 200 mg/rat 2 w (II)	Ni ore 200 mg/rat 2 w (III)	Ni ore 100 mg/rat 2 d	Ni ore 200 mg/rat 2 d
control	not done	not done	not done	not done	10.94±1.30	11.41±0.68	not done	29.61±2.32	10.94±1.30	29.72±3.82	29.72±3.82
treated					9.95±0.67	12.50±2.48		29.17±1.73	10.01±0.76	27.12±1.41	26.73±2.52
control	not done	not done	not done	not done	not done	not done	not done	not done	not done	not done	not done
treated											

Values shown are means ± S.D. of duplicate determinations from 6 individual rats per nickel ore treatment group

- \* denotes a significant difference ( $P < 0.05$ , t test)
- \*\* denotes a significant difference ( $P < 0.01$ , t test)
- \*\*\* denotes a significant difference ( $P < 0.001$ , t test)

### **3.4 *In vivo* effect of nickel subsulfide and nickel ore on rat antioxidant enzymes**

The levels of a variety of rat cytosolic enzymes were measured after nickel subsulfide or nickel ore treatment to observe the possible change of antioxidant defence systems. Soluble nickel compounds such as nickel acetate, nickel chloride, have been reported to affect the biological antioxidant systems in a strain- and time-dependent manner (Misra *et al.*, 1991; Ricardo *et al.*, 1990). The effect of insoluble nickel compounds, e.g. nickel subsulfide, upon antioxidant enzymes has not been reported.

#### **3.4.1 Catalase**

As demonstrated in Table 3.4 and Figure 3.1, a significant decrease in rat liver catalase activity occurred subsequent to nickel subsulfide treatment (200 mg/rat, single or twice dose, sacrificed in two weeks). There was a trend towards catalase inhibition in rat kidney although the decreases were not statistically significant after nickel subsulfide exposure. However, the suppression in the liver did not occur in a dose-dependent manner: nickel subsulfide 200 mg/rat (one dose, 2 w) showed a suppression of 54% while nickel subsulfide 200 mg/rat (two doses, 2 w) showed a 39% decrease.

A significant decrease occurred once in rat liver and kidney out of three identical trials in which rats were treated with nickel ore 100 mg/rat, 200 mg/rat (sacrificed in two weeks). The group treated with nickel ore 100 mg/rat (sacrificed in 2 days) showed a significant decrease in kidney but not in liver. Overall, it appears that nickel subsulfide decreases catalase activity in liver but not in kidney, whereas nickel ore does not affect catalase activity in either organ.

**Table 3.4**

The effect of nickel subsulfide and nickel ore upon catalase activity in rat liver and kidney cytosol ( $\mu\text{mol}/\text{min}/\text{mg}$  protein)

	Ni <sub>3</sub> S <sub>2</sub> 100 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 doses 2 w	Ni ore 100 mg/rat 2 w (I)	Ni ore 100 mg/rat 2 w (II)	Ni ore 200 mg/rat 1 w	Ni ore 200 mg/rat 2 w (I)	Ni ore 200 mg/rat 2 w (II)	Ni ore 200 mg/rat 2 w (III)	Ni ore 100 mg/rat 2 d	Ni ore 200 mg/rat 2 d
LC control	1085.83 ±123.20	707.96 ±102.70	606.23 ±39.24	993.65 ±162.93	761.43 ±138.84	106.92 ±15.39	993.65 ±162.93	1158.00 ±285.00	761.43 ±138.84	840.72 ±127.64	840.72 ±127.64
treated	752.93 ±314.64	327.54 ±38.23*	373.67 ±185.94*	571.06 ±117.37***	799.5 ±140.40	102.48 ±21.21	696.70 ±131.86**	964.50 ±155.00	831.22 ±153.08	755.06 ±136.56	739.2 ±170.86
KC control	362.63 ±182.75	240.69 ±83.81	289.32 ±33.73	260.15 ±58.15	341.85 ±43.97	260.15 ±23.95	260.15 ±58.15	270.50 ±32.89	341.85 ±43.97	308.61 ±82.63	308.61 ±82.63
treated	277.91 ±148.58	244.29 ±25.84	177.80 ±97.35	209.39 ±32.97	320.43 ±35.32	310.91 ±29.07	223.35 ±4.39	186.40 ±38.12**	303.78 ±35.15	207.06 ±57.26*	195.95 ±93.88

Values shown are means  $\pm$  S.D. of duplicate determinations from 3 individual rats per nickel subsulfide treatment group and 6 individual rats per nickel ore treatment group

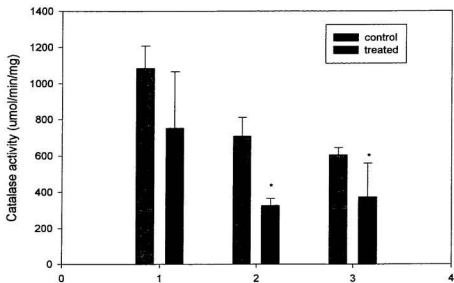
\* denotes a significant difference ( $P < 0.05$ , t test)

\*\* denotes a significant difference ( $P < 0.01$ , t test)

\*\*\* denotes a significant difference ( $P < 0.001$ , t test)

LC: liver cytosol

KC: kidney cytosol



treatment: 1: 100 mg/rat nickel subsulfide (one dose, 2 w, n=3)  
 2: 200 mg/rat nickel subsulfide (one dose, 2 w, n=3)  
 3: 200 mg/rat nickel subsulfide (two doses, 2 w, n=3)

\*  $p < 0.05$  compared with control group (t test)

**Figure 3.1 The effect of nickel subsulfide on rat liver catalase activity (umol/min/mg protein)**

### 3.4.2 Superoxide Dismutase

Misra *et al.* (1990) reported that SOD activity in male F344/NCr rat kidney and liver did not show evident changes after intraperitoneal injection of nickel acetate. In my studies, nickel subsulfide (200 mg/rat, one dose or two doses, sacrificed in 2 weeks) significantly decreased rat liver SOD activity as shown in Table 3.5 and Figure 3.2. The suppression of SOD activity was higher in the two-dose group (39.6%) than in the one-dose group (33.6%). A significant enhancement in SOD activity was observed in rat kidney only once following nickel ore treatment (200 mg/rat, as a single dose, sacrificed in 2 weeks). The rest of the experimental groups did not show any significant changes. Hence it appears that nickel subsulfide suppress SOD activity in rat liver but not in kidney. Nickel ore does not appear to affect SOD activity significantly.

**Table 3.5**  
The effect of nickel subsulfide and nickel ore on SOD activity in rat liver and kidney cytosol(unit/mg protein)

	Ni <sub>3</sub> S <sub>2</sub> 100 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 doses 2 w	Ni ore 100 mg/rat 2 w (I)	Ni ore 100 mg/rat 2 w (II)	Ni ore 200 mg/rat 1 w	Ni ore 200 mg/rat 2 w (I)	Ni ore 200 mg/rat 2 w (II)	Ni ore 200 mg/rat 2 w (III)	Ni ore 100 mg/rat 2 d	Ni ore 200 mg/rat 2 d
LC control	105.15 ±14.06	147.16 ±28.11	112.84 ±20.84	77.08 ±17.48	67.83 ±12.14	not done	77.08 ±17.48	not done	67.83 ±12.14	8.52 ±2.47	8.52 ±2.47
treated	107.86 ±12.73	97.72 ±9.97*	68.19 ±13.26*	68.70 ±4.42	74.91 ±8.99		79.85 ±11.09		70.64 ±6.01	8.53 ±0.64	7.91 ±0.51
KC control	99.07 ±30.34	50.45 ±12.98	88.47 ±10.28	61.29 ±3.73	74.99 ±8.96	not done	61.29 ±3.73	not done	74.99 ±8.96	15.05 ±3.73	15.05 ±3.73
treated	86.30 ±10.87	45.24 ±9.78	70.88 ±11.87	73.69 ±7.59	79.42 ±10.52		79.47 ±9.56*		68.18 ±7.42	14.72 ±3.26	13.82 ±2.28

Values shown are means ± S.D. of duplicate determinations from 3 individual rats per nickel subsulfide treatment group and 6 individual rats per nickel ore treatment group

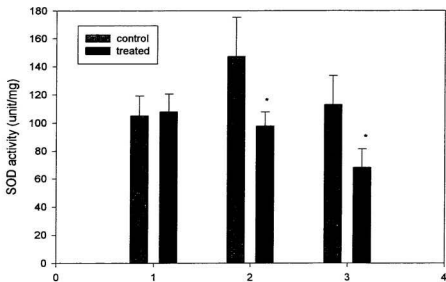
\* denotes a significant difference (P < 0.05, t test)

\*\* denotes a significant difference (P < 0.01, t test)

\*\*\* denotes a significant difference (P < 0.001, t test)

LC: liver cytosol

KC: kidney cytosol



treatment: 1: 100 mg/rat nickel subsulfide (one dose, 2 w, n=3)  
2: 200 mg/rat nickel subsulfide (one dose, 2 w, n=3)  
3: 200 mg/rat nickel subsulfide (two doses, 2 w, n=3)

\*  $p < 0.05$  compared with control group (t test)

**Figure 3.2 The effect of nickel subsulfide on rat liver SOD activity (unit/mg protein)**



### **3.4.3 Glutathione peroxidase**

The study reported by Misra *et al.* (1991) emphasized the importance of GSH and GSH-Px in preventing nickel-induced oxidative cell damage compared with CAT and SOD. As shown in Table 3.6, glutathione peroxidase activity showed a significant increase in rat liver after the treatment with nickel subsulfide (100 mg/rat, as a single dose, sacrificed in 2 weeks). Also, a significant induction in liver GSH-Px activity occurred in the group treated with nickel ore (100 mg/rat, as a single dose, sacrificed in 2 days). All other groups did not show a significant change in glutathione peroxidase activity.

**Table 3.6**

The effect of nickel subsulfide and nickel ore on glutathione peroxidase in rat liver and kidney cytosol (nmol/min/mg protein)

	Ni <sub>3</sub> S <sub>2</sub> 100 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 doses 2 w	Ni ore 100 mg/rat 2 w (I)	Ni ore 100 mg/rat 2 w (II)	Ni ore 1 w	Ni ore 200 mg/rat 2 w (I)	Ni ore 200 mg/rat 2 w (II)	Ni ore 200 mg/rat 2 w (III)	Ni ore 100 mg/rat 2 d	Ni ore 200 mg/rat 2 d
LC control	5933.61± 1285.62	7161.08± 2244.7	4775.26± 499.04	not done	2052.55± 273.30	5552.53± 692.60	not done	3023.37± 640.09	2052.55± 273.30	2863.81± 518.75	2863.81± 518.75
treated	8257.81± 1255.31*	4422.64± 419.09	4930.29± 1485.61		2290.95± 244.38	6511.87± 1385.19		3023.37± 658.83	2029.29± 266.99	3517.77± 360.31*	2965.56± 240.51
KC control	1611.69± 451.34	893.47± 213.10	1085.80± 176.97	not done	720.46± 176.94	2471.38± 251.79	not done	2805.53± 236.72	720.46± 176.94	1177.47± 211.86	1177.47± 211.86
treated	2082.6± 1275.46	721.32± 554.98	830.26± 400.96		814.03± 180.83	2500.45± 306.32		2950.90± 236.73	877.99± 122.19	1453.67± 294.06	1446.40± 369.44

Values shown are means ± S.D. of duplicate determinations from 3 individual rats per nickel subsulfide treatment group and 6 individual rats per nickel ore treatment group

\* denotes a significant difference ( $P < 0.05$ , t test)

\*\* denotes a significant difference ( $P < 0.01$ , t test)

\*\*\* denotes a significant difference ( $P < 0.001$ , t test)

LC: liver cytosol

KC: kidney cytosol

### **3.5 *In vivo* effect of nickel ore upon GSH levels in rat liver homogenate**

Administration of nickel to rats has been shown to initially reduce hepatic glutathione levels followed by an increase to levels 2 to 3 times above normal (Maines, 1980). My studies showed that no significant changes in rat liver homogenate occurred after nickel ore treatment (100 mg/rat, 200 mg/rat, sacrificed in 2 days or 2 weeks) (see Table 3.7). It appears that nickel ore does not affect rat hepatic reduced GSH levels.

**Table 3.7**  
The effect of nickel ore on reduced GSH levels in rat liver ( $\mu\text{g/g}$  liver homogenate)

	Ni <sub>2</sub> S <sub>3</sub> 100 mg/rat 2 w	Ni <sub>2</sub> S <sub>3</sub> 200 mg/rat 2 w	Ni <sub>2</sub> S <sub>3</sub> 200 mg/rat 2 doses 2 w	Ni ore 100 mg/rat 2 w (I)	Ni ore 100 mg/rat 2 w (II)	Ni ore 200 mg/rat 1 w	Ni ore 200 mg/rat 2 w (I)	Ni ore 200 mg/rat 2 w (II)	Ni ore 200 mg/rat 2 w (III)	Ni ore 100 mg/rat 2 d	Ni ore 200 mg/rat 2 d
control	not done	not done	not done	not done	95.18± 140.24	not done	not done	84.70± 11.90	95.18± 14.02	106.23± 22.07	106.23± 22.07
treated					107.67± 6.21			86.64± 11.47	105.33± 8.19	96.97± 20.51	108.20± 29.21
control	not done	not done	not done	not done	not done	not done	not done	not done	not done	not done	not done
treated											

Values shown are means  $\pm$  S.D. of duplicate determinations from 6 individual rats per nickel ore treatment group

\* denotes a significant difference ( $P < 0.05$ , t test)

\*\* denotes a significant difference ( $P < 0.01$ , t test)

\*\*\* denotes a significant difference ( $P < 0.001$ , t test)

### **3.6 *In vivo* effect of nickel ore upon glutathione reductase**

GR is one of crucial enzymes which affects cellular GSH levels because it can reduce oxidized glutathione (GSSG) to the reduced form (GSH). *In vitro* inhibition of GR by nickel chloride has been reported (Cartana *et al.*, 1989). *In vivo* studies showed quite inconsistent results. Both inhibition and enhancement have been reported due to nickel exposure (Misra, *et al.*, 1991; Athar, *et al.*, 1987). In my studies (Table 3.8), in the three similar trials with 200 mg/rat nickel ore (2 w), the results differed each time. A significant increase, a significant decrease and no effect in rat liver GR activity were all observed. The two similar trials with nickel ore 100 mg/rat (2 w) showed a significant increase once and a significant decrease once in rat liver GR activity. GR activity in rat kidney was not evidently affected. A significant increase occurred only once in rat kidney GR activity of the group treated with Ni ore 200 mg/rat (2 w). The reason for this inconsistency in the repeated trials is not clear. Overall, one must conclude that nickel ore does not induce biologically significant changes in GR activity. This observation is in keeping with the results of GSH measurement.

**Table 3.8**  
The effect of nickel ore on glutathione reductase in rat liver and kidney cytosol (nmol/min/mg protein)

	Ni <sub>2</sub> S <sub>3</sub> 100 mg/rat 2 w	Ni <sub>2</sub> S <sub>3</sub> 200 mg/rat 2 w	Ni <sub>2</sub> S <sub>3</sub> 200 mg/rat 2 doses 2 w	Ni ore 100 mg/rat 2 w (I)	Ni ore 100 mg/rat 2 w (II)	Ni ore 200 mg/rat 2 w (I)	Ni ore 200 mg/rat 2 w (II)	Ni ore 200 mg/rat 2 w (III)	Ni ore 100 mg/rat 2 d	Ni ore 200 mg/rat 2 d
LC control	not done	not done	not done	183.90 ±11.32	93.64 ±2.73	101.99 ±16.83	183.90 ±11.32	93.64 ±2.73	126.33 ±16.66	126.33 ±16.66
treated				144.98 ±5.00***	103.49 ±4.64**	108.52 ±14.95	142.46 ±12.74***	109.02 ±9.23**	118.82 ±9.17	111.39 ±8.00
KC control	not done	not done	not done	241.16 ±8.35	233.10 ±10.56	212.02 ±25.10	241.16 ±8.35	233.10 ±10.56	180.90 ±50.77	180.90 ±50.77
treated				230.71 ±21.07	246.70 ±32.26	231.11 ±15.17	233.93 ±18.21	260.20 ±17.65**	193.97 ±26.61	211.55 ±42.66

Values shown are means ± S.D. of duplicate determinations from 6 individual rats per nickel ore treatment group

\* denotes a significant difference ( $P < 0.05$ , t test)

\*\* denotes a significant difference ( $P < 0.01$ , t test)

\*\*\* denotes a significant difference ( $P < 0.001$ , t test)

LC: liver cytosol

KC: kidney cytosol

### **3.7 *In vivo* effect of nickel ore upon NAD(P)H: quinone reductase**

Studies examining the relationship between NAD(P): quinone reductase and nickel exposure were not found in the literature review. Since this enzyme is regarded as a component of biological detoxifying system, its activity after nickel ore treatment was measured and compared with the control group. As shown in Table 3.9 & Figure 3.3, nickel ore treatment of rats did not induce any significant change in rat kidney NAD(P)H: quinone reductase. In the three similar trials using 200 mg/rat nickel ore (2 w), the enzyme activity in rat liver increased significantly twice (the enhancement rate was averaged as 117%); the other group showed a trend of increase in rat liver although it was not statistically significant. In the two trials with 100 mg/rat nickel ore (2 w), the enzyme activity in rat liver increased significantly once and showed a trend of increase once although not significantly. The trials with 100 mg/rat, 200 mg/rat nickel ore (2 d) showed a decreasing trend but this was not significant. Significant effects were not observed in rat kidney following nickel ore treatment. Overall, it appears that nickel ore is able to increase rat liver NAD(P)H: quinone reductase activity in a time-dependent manner.

**Table 3.9**

The effect of nickel ore on NAD(P)H: quinone reductase in rat liver and kidney cytosol (nmol/min/mg protein)

	Ni <sub>2</sub> S <sub>3</sub> 100 mg/rat 2 w	Ni <sub>2</sub> S <sub>3</sub> 200 mg/rat 2 w	Ni <sub>2</sub> S <sub>3</sub> 200 mg/rat 2 doses 2 w	Ni ore 100 mg/rat 2 w (I)	Ni ore 100 mg/rat 2 w (II)	Ni ore 200 mg/rat 1 w	Ni ore 200 mg/rat 2 w (I)	Ni ore 200 mg/rat 2 w (II)	Ni ore 200 mg/rat 2 w (III)	Ni ore 100 mg/rat 2 d	Ni ore 200 mg/rat 2 d
LC control	not done	not done	not done	104.56 ±24.48	160.65 ±76.5	not done	104.56 ±24.48	279.18 ±13.25	160.65 ±76.50	285.71 ±76.16	285.71 ±76.16
treated				164.57 ±48.70*	219.25 ±48.53		224.35 ±38.21***	320.84 ±125.59	352.60 ±137.16*	277.38 ±23.33	251.79 ±77.09
KC control	not done	not done	not done	26.68 ±2.51	36.59 ±19.72	25.30 ±8.94	26.68 ±2.51	56.88 ±18.28	36.59 ±19.72	35.41 ±5.69	35.41 ±5.69
treated				25.48 ±9.51	32.28 ±3.11	24.30 ±3.77	31.19 ±10.31	42.15 ±12.76	48.35 ±18.00	40.17 ±5.29	41.65 ±9.61

Values shown are means ± S.D. of duplicate determinations from 6 individual rats per nickel ore treatment group

\* denotes a significant difference ( $P < 0.05$ , t test)

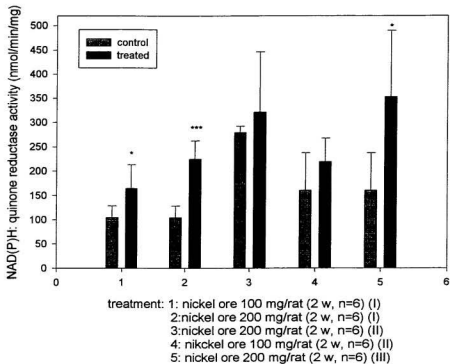
\*\* denotes a significant difference ( $P < 0.01$ , t test)

\*\*\* denotes a significant difference ( $P < 0.001$ , t test)

LC: liver cytosol

KC: kidney cytosol





\*  $p < 0.05$  compared with control group (t test)

\*\*\*  $p < 0.001$  compared with control group (t test)

**Figure 3.3 The effect of nickel ore on rat liver NAD(P)H: quinone reductase activity (nmol/min/mg protein)**

### **3.8 *In vivo* effect of nickel ore upon glutathione S-transferases**

The differential responses among the substrates of hepatic GSTs to a great variety of xenobiotics have been well established (Iscan, 1993). Rather contradictory results in the literature have been reported regarding the GST response to nickel exposure. Enhancement and inhibition were both observed (Athar, 1987; Misra, 1990). The reason for the differences observed among these studies may be attributable to differences in the age, strain and nutritional status of the animals used (Iscan, 1993).

#### **3.8.1 With CDNB as substrate**

CDNB is the most important substrate for the demonstration of multiple forms of GST (Iscan, 1993). As shown in Table 3.10 and Figure 3.4, a significant decrease in rat liver GST activity (with CDNB as substrate) occurred in the nickel ore treated group (100 mg/rat, as a single dose, sacrificed in 2 weeks) but only once in repeated trials. GST activity increased significantly in rat kidney due to the treatment of nickel ore (both 100 & 200 mg/rat group, as a single dose, sacrificed in 2 days). It seems that nickel ore treatment could increase GST activity (CDNB) in rat kidney shortly after treatment (2 d). The treatment of rats with nickel ore for 1 week or 2 weeks prior to sacrifice does not have any evident effect on GST activity (CDNB).

**Table 3.10**

The effect of nickel ore on glutathione S- transferase (CDNB) activity in rat liver and kidney cytosol

LC:  $\mu\text{mol}/\text{min}/\text{mg}$  protein

KC:  $\text{nmol}/\text{min}/\text{mg}$  protein

	Ni <sub>3</sub> S <sub>2</sub> 100 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 doses 2 w	Ni ore 100 mg/rat 2 w (I)	Ni ore 100 mg/rat 2 w (II)	Ni ore 200 mg/rat 1 w	Ni ore 200 mg/rat 2 w (I)	Ni ore 200 mg/rat 2 w (II)	Ni ore 200 mg/rat 2 w (III)	Ni ore 100 mg/rat 2 d	Ni ore 200 mg/rat 2 d
LC control treated	not done	not done	not done	2.40 $\pm$ 0.30	2.65 $\pm$ 0.15	8.75 $\pm$ 1.24	2.40 $\pm$ 0.30	2.58 $\pm$ 3.19	2.65 $\pm$ 0.15	2.18 $\pm$ 0.32	2.18 $\pm$ 0.32
				1.82 $\pm$ 0.24**	2.64 $\pm$ 0.32	9.17 $\pm$ 1.13	2.02 $\pm$ 0.26	2.32 $\pm$ 4.03	2.62 $\pm$ 0.16	2.32 $\pm$ 0.35	2.09 $\pm$ 0.15
KC control treated	not done	not done	not done	427.08 $\pm$ 72.59	612.27 $\pm$ 73.01	1458.66 $\pm$ 98.54	427.08 $\pm$ 72.59	639.12 $\pm$ 62.86	612.27 $\pm$ 73.01	437.80 $\pm$ 72.13	437.80 $\pm$ 72.13
				429.69 $\pm$ 28.17	618.78 $\pm$ 121.72	1387.16 $\pm$ 253.99	473.96 $\pm$ 82.80	587.05 $\pm$ 32.47	552.05 $\pm$ 34.20	556.61 $\pm$ 98.43*	568.00 $\pm$ 113.6*

Values shown are means  $\pm$  S.D. of duplicate determinations from 6 individual rats per nickel ore treatment group

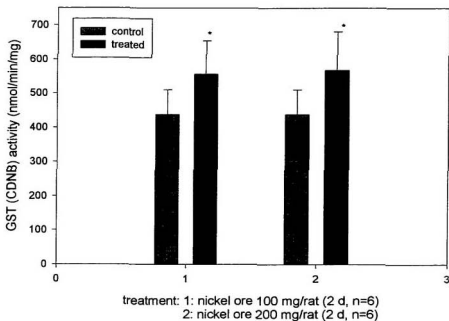
\* denotes a significant difference ( $P < 0.05$ , t test)

\*\* denotes a significant difference ( $P < 0.01$ , t test)

\*\*\* denotes a significant difference ( $P < 0.001$ , t test)

LC: liver cytosol

KC: kidney cytosol



\*  $p < 0.05$  compared with control group (t test)

**Figure 3.4 The effect of nickel ore on rat kidney GST (CDNB) activity (nmol/min/mg protein)**

### **3.8.2 With DCNB as substrate**

As shown in table 3.11, nickel ore treatment did not cause any significant changes in rat liver and kidney GST (DCNB) activity. This observation is in keeping with the study reported by Iscan *et al.*(1993).

**Table 3.11**

The effect of nickel ore on glutathione S-transferase (DCNB) activity in rat liver and kidney (nmol/min/mg protein)

	Ni <sub>3</sub> S <sub>2</sub> 100 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 doses 2 w	Ni ore 100 mg/rat 2 w (I)	Ni ore 100 mg/rat 2 w (II)	Ni ore 200 mg/rat 1 w	Ni ore 200 mg/rat 2 w (I)	Ni ore 200 mg/rat 2 w (II)	Ni ore 200 mg/rat 2 w (III)	Ni ore 100 mg/rat 2 d	Ni ore 200 mg/rat 2 d
LC control treated	not done	not done	not done	112.94 ±9.73  101.76 ±12.71	164.15 ±24.05  169.15 ±43.38	95.53 ±11.86  102.20 ±9.43	112.94 ±9.73  108.24 ±18.36	141.20 ±13.38  122.82 ±17.74	164.15 ±24.05  158.26 ±15.05	78.55 ±11.64  86.20 ±14.23	78.55 ±11.64  80.98 ±5.64
KC control treated	not done	not done	not done	1.82 ±0.20  2.11 ±0.54	not done	not done	1.82 ±0.20  1.97 ±0.22	not done	not done	not done	not done

Values shown are means ± S.D. of duplicate determinations from 6 individual rats per nickel ore treatment group

LC: liver cytosol

KC: kidney cytosol

### **3.8.3 Ethacrynic acid**

As shown in Table 3.12, nickel ore treatment did not cause any significant changes in rat liver & kidney GST (ethacrynic acid) activity. Iscan *et al.* (1992 and 1993) reported a increase in GST (EAA) after nickel chloride treatment in rat liver. However, we should keep in mind that insoluble nickel compounds may act differently from soluble ones.

**Table 3.12**

The effect of nickel ore on glutathione S-transferase (ethacrynic acid) activity in rat liver and kidney cytosol (nmol/min/mg protein)

	Ni <sub>3</sub> S <sub>2</sub> 100 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 doses 2 w	Ni ore 100 mg/rat 2 w (I)	Ni ore 100 mg/rat 2 w (II)	Ni ore 200 mg/rat 1 w	Ni ore 200 mg/rat 2 w (I)	Ni ore 200 mg/rat 2 w (II)	Ni ore 200 mg/rat 2 w (III)	Ni ore 100 mg/rat 2 d	Ni ore 200 mg/rat 2 d
LC control	not done	not done	not done	53.00 ±3.99	26.70 ±7.82	52.38 ±7.67	53.00 ±3.99	23.25 ±5.38	26.70 ±7.82	36.88 ±2.86	36.88 ±2.86
treated				52.00 ±3.76	27.50 ±7.37	54.88 ±3.84	56.50 ±3.76	22.50 ±12.77	30.38 ±11.49	37.38 ±2.74	37.38 ±3.23
KC control	not done	not done	not done	42.00 ±3.00	60.2 ±3.32	43.65 ±4.18	42.00 ±3.00	not done	60.20 ±3.32	42.00 ±4.14	42.00 ±4.14
treated				45.00 ±4.91	57.7 ±8.53	42.65 ±5.83	45.00 ±3.18		62.70 ±5.13	45.00 ±9.90	46.00 ±2.63

Values shown are means ± S.D. of duplicate determinations from 6 individual rats per nickel ore treatment group

\* denotes a significant difference ( $P < 0.05$ , t test)

\*\* denotes a significant difference ( $P < 0.01$ , t test)

\*\*\* denotes a significant difference ( $P < 0.001$ , t test)

LC: liver cytosol

KC: kidney cytosol



#### **3.8.4 With trans-4-phenyl-3-buten-3-one as substrate**

As shown in Table 3.13, the changes in rat liver GST (trans-4-phenyl-3-buten-3-one) activity were not consistent in three similar trials with nickel ore (200 mg/rat, as a single dose, sacrificed in 2 weeks). A significant increase, a significant decrease and no significant change in rat liver GST activity (trans-4-phenyl-3-buten-3-one) were all observed. Kidney GST (trans-4-phenyl-3-buten-3-one) activity did not show any significant changes following the treatment with nickel ore. In general, nickel ore treatment appeared to have no effect upon GST (trans-4-phenyl-3-buten-3-one) activity.

**Table 3.13**

The effect of nickel ore on glutathione S-transferase (trans-4-phenyl-3-buten-3-one) activity in rat liver and kidney cytosol (nmol/min/mg protein)

	Ni <sub>3</sub> S <sub>2</sub> 100 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 w	Ni <sub>3</sub> S <sub>2</sub> 200 mg/rat 2 doses 2 w	Ni ore 100 mg/rat 2 w (I)	Ni ore 100 mg/rat 2 w (II)	Ni ore 200 mg/rat 1 w	Ni ore 200 mg/rat 2 w (I)	Ni ore 200 mg/rat 2 w (II)	Ni ore 200 mg/rat 2 w (III)	Ni ore 100 mg/rat 2 d	Ni ore 200 mg/rat 2 d
LC control treated	not done	not done	not done	20.62 ±5.91  15.23 ±2.40	21.70±8.26  21.82±5.58	21.42 ±1.49  22.56 ±3.86	20.62 ±5.91  14.52 ±2.41*	17.77 ±2.62  15.81 ±1.70	21.70±8.26  31.75±5.97*	15.29±2.20  15.48±2.59	15.29±2.20  14.54±1.80
KC control treated	not done	not done	not done	0.93 ±0.32  0.80 ±0.10	1.26±0.38  1.41±0.54	not done	0.93 ±0.32  0.64 ±0.14	not done	1.26±0.38  0.98±0.28	not done	not done

Values shown are means ± S.D. of duplicate determinations from 6 individual rats per nickel ore treatment group

\* denotes a significant difference ( $P < 0.05$ , t test)

\*\* denotes a significant difference ( $P < 0.01$ , t test)

\*\*\* denotes a significant difference ( $P < 0.001$ , t test)

LC: liver cytosol

KC: kidney cytosol

## CHAPTER 4

### 4. DISCUSSION

#### 4.1 *In vivo* effects of nickel subsulfide

The mechanism of nickel toxicity and carcinogenicity has gained some clarification in recent years. Numerous studies regarding the toxicity and carcinogenicity of nickel subsulfide have been conducted. With regard to carcinogenicity assessment, nickel subsulfide has been suggested as the Ni compound which presents the highest carcinogenic potential relative to other Ni compounds (Oller *et al.*, 1997). *In vivo*, nickel subsulfide is likely to be readily endocytized and dissolved by the target cells resulting in efficient delivery of  $\text{Ni}^{2+}$  (Costa and Mollenhauer, 1980). However, studies on the *in vivo* effect of nickel subsulfide on oxidative damage to lipid (e.g. lipid peroxidation) and protein (e.g. protein carbonyls formation) and antioxidant defence systems are few (Rodriguez *et al.*, 1996; Teissier *et al.*, 1994; Shirali *et al.*, 1994; Sunderman *et al.*, 1985; Benson<sup>b</sup> *et al.*, 1986). Most of the studies were performed with water soluble nickel compounds.

*In vivo* effects of nickel subsulfide on rat antioxidant defence systems (e.g. catalase, SOD, GSH-Px) were investigated in my research. An overall review of the results led to the conclusion that nickel subsulfide seems to be able to suppress catalase and SOD activity in rat liver but not kidney (Table 3.4, Figure 3.1, Table 3.5, Figure 3.2). Inhibition of catalase did not show a dose-dependent relationship, whereas inhibition in SOD showed a 34% suppression for the one-dosage group and a 40% suppression for the two-dosage group. It also appears that nickel subsulfide does not exert a significant effect upon GSH-Px.

According to Oller *et al.* (1997), nickel subsulfide could be efficient at promoting both heritable changes and cell proliferation during its carcinogenesis. The involvement of oxygen radicals generated through a Ni(III)/Ni(II) redox mechanism could induce damaged bases, DNA strand breaks, DNA-protein crosslinks and chromosomal aberrations (Oller *et al.*, 1997). Huang *et al.* (1994) reported that nickel subsulfide increased the concentration of oxidants in CHO cells. Hydrogen peroxide is one of the formed oxidants in this system. Lipid hydroperoxides as products of induced lipid peroxidation may also be produced through the action of nickel in intact cells. It is known that catalase, SOD and GSH-Px appear to work in conjunction minimizing the cell's exposure to reactive intermediates of oxygen reduction (Misra *et al.*, 1990). Hence the diminishment of hepatic CAT and SOD by nickel subsulfide in my studies could be a reflection of nickel insult which would make target tissues more vulnerable under oxidative stress.

It is assumed that the toxicity elicited by nickel is in close relationship with nickel concentration in target tissues. However, this is not always the case. In the report by Misra *et al.* (1990), the magnitude of LPO was not proportional to the nickel concentration (nickel acetate) in the respective organ. Both liver and kidney showed the same LPO increase level (110-130%) while the concentration of nickel in kidney was 10 fold higher than in liver. In my studies, no evident antioxidant defence system changes were showed in rat kidney which supposedly is the main excreting organ and hence possessing a higher concentration of nickel. The exact reason for this phenomenon is not clear. One possibility for this difference could

be the fact that very little of the nickel subsulfide had dissolved and hence the concentrations in the kidney (as well as other organs) ready for excretion, were low.

It was reported that microsomal heme oxygenase activity in rat kidney increased 1.5 fold one week after intrarenal injection of 10 mg/rat nickel subsulfide. At 2, 3, 4 weeks after intrarenal injection of  $\text{Ni}_3\text{S}_2$ , renal heme oxygenase activities did not differ significantly from the corresponding values in control rats (Sunderman *et al.*, 1983). Heme constitutes the prosthetic moiety of a variety of hemoproteins. The list includes catalase, several peroxidases and a large number of cytochromes P450. A perturbation of heme metabolism may be involved in the changes in rat hepatic catalase and GSH-Px activity after nickel subsulfide administration. However, further investigations will be needed to prove this.

The mode of nickel subsulfide action could be attributed to its metabolism kinetics after administration. It was reported that after parenteral treatment of  $^{63}\text{Ni}_3\text{S}_2$  to rats, the excretory half-time of  $^{63}\text{Ni}$  released from  $^{63}\text{Ni}_3\text{S}_2$  averaged 24 days; Ni (II) slowly dissolved in body fluids and was excreted in the urine (Kuehn and Sunderman, 1982 ).

#### **4.2 *In vivo* effect of nickel ore in rat liver and kidney**

The nickel ore sample used in my research was obtained from PCMR CANMET. The relative element content in the sample is as follows:

Ni, 1.233 wt%; Cu, 0.967 wt%; Co 0.041 wt%; Pt, 0.41 ug/g; Pd, 0.37 ug/g; Ag, 4.30 ug/g.

It is worth noting that nickel ore consists of a mixture of metals and the response of organisms to it may be quite different to the response of organisms to pure metal compounds that are used in most studies. Since nickel and copper are the dominant components in the

sample, the final toxic response to nickel ore might be due to the synergistic or antagonistic action between Ni and Cu. However, this does not rule out the effects exerted by the other metals. Also, the manner in which nickel is complexed in the ore and its solubility (or lack of it) will have a significant impact on how it affects organisms. In this regard, the effects of nickel ore may resemble those of nickel subsulfide since the latter is also insoluble.

From the overall review of the results of nickel ore, it appears that nickel ore does not affect LPO, protein carbonyl content, ALA dehydratase, CAT, SOD, GSH-Px, GSH, GR, GST (DCNB, EAA, trans-4-phenyl-3-buten-3-one) in rat liver or kidney to any significant extent. A great variation in the activities of SOD and GR were observed between the various control groups and so in replicate experiments. The reason for this variation is not clear. Samples from both control and treated groups within a single experiment were analyzed on the same day using the same batch of reagents. However, samples from different experiments were analyzed at different times using freshly made reagents each time. This should not normally lead to large differences in activity as was observed in some instances. In the case of replicate experiments, where a statistical significance was observed, it was marginal and was not seen in repeat experiments possibly due to high standard deviation. A statistically significant increase was observed in liver NAD(P)H: quinone reductase activity in the group treated with 200 mg/rat (2 w); the enhancement rate averaged 117%. An increase also occurred in kidney GST(CDNB) activity in the groups treated with 100 and 200 mg/rat (2 d) and this activation seemed to be induced in a dose-dependent manner.

LPO and protein carbonyl levels in biological tissues represent oxidative damage to

lipid and protein. The lack of alteration of these two parameters after nickel ore administration suggests that the oxidative damage is not involved in the toxicity response of rats to nickel ore exposure under the specific dosage and duration regimens set in my research. However, this does not rule out the possibility that oxidative damage may be evident under other circumstances (e.g. different dosage or duration prior to sacrifice). It has been established that there is a direct relationship between LPO and tissue iron and copper content, involving reactive oxygen species. Nickel treatment may also assist in generation of activated oxygen species (Misra *et al.*, 1990). It is quite reasonable to assume that an increase in LPO levels could result from the combination Ni and Cu (e.g. nickel ore), especially since copper is known to redox cycle between Cu (II) and Cu (I) (Patel *et al.*, 1997). However, this was not the case. The reason could be attribute to low concentration of nickel or copper in the kidney and liver because of the low solubility of nickel or copper complex in the ore.

According to the report by Misra *et al.* (1990), three hours after intraperitoneal administration of nickel acetate to male F 344/NCr rats there were increased LPO levels, decreased CAT and GSH-Px activity, decreased GSH levels and decreased GR activity in both liver and kidney. The concurrence of LPO increase and decrease in the activity of H<sub>2</sub>O<sub>2</sub>-scavenging enzymes (e.g. CAT and GSH-Px) could augment the potential cellular oxidative damage. The tissue GSH level fluctuates with the levels of GSH synthesis and metabolism enzymes (e.g. GR, GSH-Px, GST). Interaction of metals with glutathione metabolism is an integral part of the toxic response of many metals (Cartana *et al.*, 1992). It is known that GSH plays several important roles which include the maintenance of sulfhydryl enzymes,

integrity of membrane against peroxidative damage, regulation of amino acid transport and maintenance of intracellular redox potential (Athar *et al.*, 1987). Copper could produce a drastic drop of the GSH/GSSG ratio in both liver and kidney. Renal GST inhibition and hepatic GR inhibition was also observed (Cartana *et al.*, 1992). My results indicate that the effect of nickel ore on the antioxidant defence systems is not significant. This result is in keeping with my observations that LPO and protein carbonyl content are not changed. This may be due to the difference in action between soluble and insoluble nickel compounds. Again, different routes of administration, nutritional state and the test compound used could explain the discrepancies.

Nickel exposure could also cause ALA dehydratase inhibition thus inducing a perturbation in heme metabolism (Maines, 1980). Also, heme is the main constituent of many detoxifying enzymes, e.g. CAT, peroxidases and cytochrome P450. The lack of change in ALA dehydratase is in keeping with the lack in CAT and GSH-Px activities after nickel ore exposure.

Interestingly, a significant increase was observed in kidney GST (CDNB) in the groups treated with 100 and 200 mg/rat (2 d). The activation seemed to be induced in a dose-dependent manner. The GSTs are a group of multifunctional enzymes, with broadly overlapping substrate specificities, which are generally involved in the detoxification of the activated electrophilic xenobiotics (Iscan *et al.*, 1992; Iscan *et al.*, 1993). GST activities for DCNB, EAA and trans-4-phenyl-3-buten-3-one are relatively specific measures of rat liver isozymes 3-3, 7-7 and 4-4. CDNB is the most important substrate for the demonstration of



multiple forms of GST (Mannervik and Danielson, 1988). Administration of nickel chloride subcutaneously 16 hr prior to sacrifice was reported to increase hepatic GST (EAA) while exerting no effect on GST(CDNB and DCNB) (Iskan *et al.*, 1992). Administration of nickel acetate intraperitoneally 3 hr prior to sacrifice was reported to increase kidney GST (CDNB) by 44% ( Misra *et al.*, 1990). In the current study, the observed enhancement of GST (CDNB) activity and the lack of alteration GST (DCNB, EEA and trans-4-phenyl-3-buten-3-one) activity was good manifestation for the differential regulation of isozymes of GSTs by Ni in rat liver and kidney. It is noteworthy that copper could induce a considerable inhibition in kidney GST (CDNB) activity (Cartana *et al.*, 1992). The response of rats in GSTs may be due to the combination effect of nickel and copper in nickel ore. The increase in GST activity suggests the ability of rat kidney to cope with the insult of nickel ore by increasing its detoxifying capacity.

NAD(P)H: quinone reductase is often regarded as a phase II detoxification system. It is suggested that this enzyme could help maintain coenzyme Q in the reduced form hence recycling it as an inhibitor of lipid peroxidation (Halliwell and Gutteridge, 1999). In the current study, a statistically significant increase was observed in liver NAD(P)H: quinone reductase activity in the group treated with 200 mg/rat (2 w); the enhancement rate averaged 117%. This could be regarded as an increase in the liver detoxifying capacity under nickel ore insult.

Overall, nickel subsulfide suppressed antioxidant defence enzymes by inhibiting hepatic CAT and SOD. Nickel ore generally did not cause substantial changes although

kidney GST (CDNB) and renal NAD(P)H: quinone reductase activities were increased. It appears that nickel ore does not pose an evident toxic insult to rats in short term studies and hence the risk posed to aquatic species may be minimal based on these studies. However, long-term studies using rainbow trout and bivalves need to be conducted to investigate possible risks of more broadscale environmental concern.

## CHAPTER 5

### 5. CONCLUSION

- Nickel subsulfide administration (200 mg/rat, single or twice dose, 2 w) suppressed hepatic CAT and SOD which could compromise the biological antioxidant system.
- Nickel ore does not affect LPO, protein carbonyl content, ALA dehydratase, CAT, SOD, GSH-Px, GSH, GR, GST (DCNB, EAA, trans-4-phenyl-3-buten-3-one) in rat liver and kidney to any significant extent.
- Nickel ore induced a statistically significant increase in liver NAD(P)H: quinone reductase activity in the group treated with 200 mg/rat (2 w). A significant increase occurred in kidney GST(CDNB) in the groups treated with 100 and 200 mg/rat (2 d) and the activation seemed to be dose-dependent. The increase in these activities could help boost the protective capacity of the rats.
- It appears that nickel ore does not pose an evident toxic insult to rats in short term studies and hence the risk posed to aquatic species could be minor. However, further studies with rainbow trout need to be conducted to investigate possible risks of more broadscale environmental concern:
  - It is proposed to prepare tailings from the authentic ore and analyze its uptake and potential toxicity. Short-term and long-term *in vivo* studies in rainbow

trout will be carried out. Trout will be either injected (i.p.) by a suspension of tailings or exposed to diluted leachate in 300 L tanks for a period of 1 to 2 weeks. Tailings with particle size less than 5 microns will also mixed with sand at the bottom of the tank to observe the effect after 1 to 4 months. Livers and kidneys will be removed and aliquots processed to measure lipid peroxidation, glutathione levels, oxidant and antioxidant enzymes. Cu and Ni content in the tissues will be measured as well. Neutrophils will be isolated to measure the respiratory burst. Differential blood cell counts will be measured as an indication of immunologic changes.

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